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BRIEFER ARTICLES

THE BASIDIUM OF AMANITA BISPORIGERA.[1]

(WITH SEVENTEEN FIGURES)

THERE are among the Hymenomycetes certain species which have basidia bearing only two spores instead of the usual four. Such a form is sometimes found in a genus the other members of which have basidia with the usual number of spores. Some time ago while studying the structure of a white Amanita which resembles *A. verna*, Professor ATKINSON discovered that certain of the plants had basidia with only two spores. He also found that the two-spored plant could be distinguished from the four-spored *A. verna* by other characters, and he has described it as a new species, *A. bisporigera.*

AMANITA BISPORIGERA.

Amanita bisporigera Atkinson, n. sp.—Plants entirely white, usually occurring singly, about 9–13^{cm} high; pileus 4–6.5^{cm} broad; stem 5–8^{mm} thick; bulb 2–2.5^{cm} thick. Pileus convex to expanded, thin, often gibbous or somewhat broadly umbonate, smooth, viscid when moist, thin. Gills subelliptical, usually tapering more behind where they are rounded and free but close, rather crowded, edge of gills floccose. Basidia 2-spored. Spores globose to subglobose or oboval, smooth, with a minute pedicel where attached to the sterigmata as in many species, 8–10 μ. Stem nearly even, or slightly tapering upward, solid, when fresh finely floccose scaly both above and below the annulus, in age tending to become smooth especially below the annulus. Annulus thin, membranous, fragile, sometimes entire, sometimes torn, superior. Volva thick, with apical dehiscence and with the free limb splitting into two or three lobes which are usually closely appressed against the stem.

On the ground in woods. It has been found many times at Ithaca, N. Y. and vicinity, and specimens under twelve or more collections are in the Herbarium of the Department of Botany, Cornell University.

The plant bears a striking resemblance to *A. verna*, but is distinguished by its more slender habit and the two-spored basidia.

As this plant so closely resembles a four-spored species, it seemed desirable to study the nuclear phenomena in the basidium to determine how the behavior of the nuclei compares with what has been observed in the four-spored forms by WAGER (5, 6), JUEL (4), HARPER (2), and others. The results of a number of investigators make it seem very probable that in all Basidiomycetes the young basidium contains two small primary nuclei which fuse to form the secondary nucleus of the basidium.

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[1] Contributions from the Department of Botany, Cornell University, No. 108.

Wager, however, was of the opinion that in some cases more than two nuclei move into the young basidium from the multinucleate cells of the hyphae. After the fusion of the primary nuclei, the secondary nucleus increases greatly in size. By two successive divisions of this nucleus, four nuclei are produced for the spores. In basidia with more than four spores, as in some Gastromycetes, there are probably more than two divisions. In Dacryomyces, which has basidia with only two spores, there has been some difference of opinion, but it seems probable that the two nuclear divisions take place in the usual way. Dangeard (1) observed only one division in *D. deliquescens* Bull. Istvánffi (3) found that in *D. chrysocomus* Bull. there are two successive divisions, and the four nuclei arrange themselves in a row and move in pairs into the branches of the basidium.

However, the young spore receives but one nucleus, as one remains behind in each branch of the basidium. Juel (4) worked with *D. deliquescens* Bull. and found that there are two successive divisions of the nucleus, but each spore receives a single nucleus, the others remaining behind in the basidium.

The material for this study was secured during the summer of 1904 from plants collected in the vicinity of Ithaca, N. Y. Small pieces were cut from the pilei of young plants soon after their collection and were placed in 1 per cent. chromacetic acid where they remained 12–24 hours. The material was then washed 3–4 hours in running water, dehydrated in grades of alcohol, and passed gradually into paraffin. Sections were cut 3–5 μ in thickness. In sections showing some mature spores several stages in the development of the basidium are found. It is better, however, for the study of the young stages to take sections from younger plants. In fixing material from plants so young that none of the basidia bear sterigmata, there is a possibility of confusing these plants with *A. verna*. To avoid this, small pieces were cut from the pilei of the young plants and these plants were then placed in a moist chamber and allowed to continue their growth until the spores were produced. Then by freehand sections of the pileus the species could be exactly determined. *A. bisporigera* is so distinct in appearance, however, on account of its more slender form, that after one becomes familiar with it there is no difficulty in distinguishing it from *A. verna*, even before it is fully mature.

The sections were stained with safranin and gentian violet, which gives very good results. The preparations were studied with Zeiss 2^{mm} apochromatic, 1.40 aperture, and oculars 8, 12, and 18. Drawings were made with camera lucida and ocular 18.

Material of the two-spored *Agaricus campestris* was prepared for study in the same way, but on account of the smaller size of the nuclei and the dense contents of the basidium, this plant does not offer favorable material.

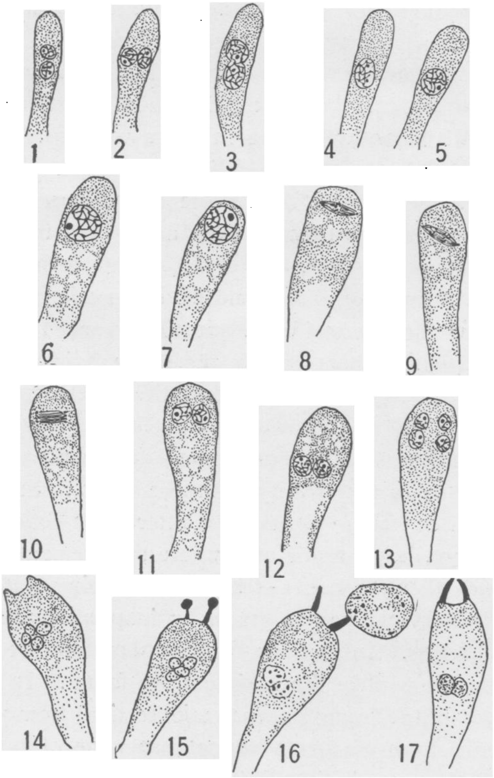

FIG. 1. Young basidium which is densely filled with cytoplasm and contains two primary nuclei.—FIG. 2. The membranes of the two nuclei are in contact.—FIG. 3. Two primary nuclei in the process of fusion, but the nucleoli indistinct.—FIG. 4. Fusion of the nuclei almost complete.—FIG. 5. Basidium somewhat increased in size after fusion of primary nuclei.—FIGS. 6 and 7. Older basidia in which the secondary nucleus occupies a position near the end of the basidium; structure of nucleus at this stage very distinct.—FIG. 8. Division of secondary nucleus.—FIG. 9. Chromosomes moving to the poles of the spindle.—FIG. 10. Chromosomes at poles of the spindle.—FIGS. 11 and 12. Daughter nuclei occupying different positions in basidium; usually near the end.—FIG. 13. Basidium showing four nuclei.—FIG. 14. The four nuclei crowded together at some distance from the end of the basidium and the sterigmata beginning to form.—FIG. 15. The cytoplasm beginning to pass through the sterigmata to form the spores.—FIG. 16. A basidium in which the spores are almost mature; two nuclei still seen near the center of the basidium.—FIG. 17. Old basidium from which spores have fallen; two nuclei near the center.

The young basidium first appears as a club-shaped branch from the sub-hymenial layer. It is more densely filled with protoplasm than in older stages and contains two nuclei. The structure of these nuclei can be made out very easily. Each nucleus has a rather large deeply staining nucleolus, a network in which the chromatin granules are imbedded, and a distinct nuclear membrane.

As the basidium increases in size the nuclei fuse together into one large nucleus. At first the two nuclei lie side by side with their membranes in contact and without apparent change. Then the membranes disappear at the point of contact. The nucleoli remain distinct for a short time, but finally fuse so that the large secondary nucleus produced by the fusion contains but a single nucleolus. The manner of the fusion of the threads bearing the chromatin could not be made out. After this fusion, the nucleus increases in size and comes to occupy a position near the upper expanded end of the basidium (*figs. 6* and *7*).

At this stage the structure of the nucleus can best be determined. It is so large that it fills more than two-thirds the diameter of the basidium. The nuclear membrane is very distinct, so that the nucleus stands out very clearly from the contents of the basidium, which at this stage have become vacuolate. The nucleolus is large and stains deeply, taking a reddish color with the triple stain. The nuclear network consists of one or more coiled threads in which are imbedded the chromatin granules, which stain blue or purple. Between the coils of the thread are the colorless spaces which are filled by the nuclear sap in the living cell.

The nuclear division takes place in the manner described by WAGER (5) for *A. muscaria.* The chromosomes are produced from the thread bearing the chromatin granules. The number of chromosomes is small, but I have been unable to determine the exact number. The chromosomes are small and stain deeply. The spindle consists of a small number of fibers, but they do not show the structures of fibers very clearly because they are crowded closely together. The spindle is arranged transversely near the apex of the basidium and is long and narrow with a small deeply staining body at each pole. Such spindles as the one shown in *fig. 8* are found frequently in the preparations. After the chromosomes move to the poles there are a few persisting spindle fibers which connect them. The daughter nuclei are now formed and usually occupy a position near the apex of the basidium (*fig. 11*). Each daughter nucleus has the same form and structure as the parent nucleus.

The most important question now is whether these two nuclei divide again to produce four nuclei, as is the case in plants with four spores on a basidium. After careful search I have been unable to find the spindles of such a division, but numerous basidia which contained four nuclei have been observed. In some cases only three nuclei show in the section, but it seems probable that in all such cases the fourth nucleus is in another section. These nuclei are small and in most cases the structure is not so distinct as in the earlier nuclei of the basidium (*fig. 13*). The four nuclei move back from the apex of the basidium and become crowded together in an irregular mass in which it is difficult to distinguish the individual nuclei (*figs. 14* and *15*).

Soon after the four nuclei are formed, the two sterigmata grow out from the end of the basidium. The granular content of the basidium moves up and becomes more dense near the apex. Then the protoplasm begins to pass out through the sterigmata to form the spores. The question which now presents itself is as to the number of nuclei which pass into the spores. It is difficult to follow the details of the passage of the nucleus through the sterigma, and it seems to me that the best evidence as to the number of nuclei which enter the spores is found in the examination of old basidia in which the spores are fully mature or may have fallen off. Such basidia which contain only a small amount of cytoplasm show the presence of two nuclei (*fig. 17*). From the facts that the basidium when the sterigmata are formed contains four nuclei and that it contains only two when the spores are mature one may conclude that two nuclear divisions take place as in those forms with basidia which bear four spores, but that only two of the nuclei enter the spores.—CHARLES E. LEWIS, *Cornell University*.

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